Original Article

Can Leukocyte Telomere Length Predict Survival Time in Heart Transplant Recipients over a Minimal Follow-Up of 20 years?

(leukocyte telomere length / survival time / heart transplant)

D. DLOUHÁ[1], V. VANČURA[2,3], J. VYMĚTALOVÁ[3], J. A. HUBÁČEK[1], V. LÁNSKÁ[4], I. MÁLEK[3]

[1]Centre for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
[2]Cardiology Department, Complex Cardiovascular Centre, Charles University, Faculty of Medicine Pilsen and University Hospital in Pilsen, Czech Republic
[3]Cardiology Centre, [4]Medical Statistical Unit, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Abstract: In humans, leukocyte telomere length (LTL) reduces with age and is reported to be inversely associated with ageing-related diseases. We measured LTL in leukocyte DNA using a quantitative PCR-based method from 127 blood samples of heart recipients (107 males, 20 females, age 44.1 ± 10.5), followed for up to 30 years. Patients with coronary artery disease survived for a shorter time and also had shorter LTL (both P < 0.05 after adjustment for age and sex) than subjects with dilated cardiomyopathy. Patients with non-cardiac causes of death had shorter LTL than patients with cardiac causes (P < 0.05 after adjustment for age). An inverse correlation between LTL and age (P < 0.03) was observed in patients with non-cardiac causes of death only. Most importantly, LTL was not associated with general survival time in patients after heart transplantation. However, shorter LTL was a marker of non-cardiac causes of death. Different LTLs and survival times were determined in association with aetiology of heart failure (HF).

Introduction

Although the first heart transplantation was performed almost 50 years ago and there has been clear progress in recent decades leading to thousands of transplantations successfully performed every year around the world, there is still a significant lack of molecular markers that allow survival to be predicted in these patients.

One possible mechanism of the ageing heart is cellular senescence. Leukocyte telomere length (LTL) is a marker of replicative ageing. Telomeres are composed of repetitive sequences of six bases (TTAGGG) located at the ends of chromosomes in eukaryotic cells. These sequences are repeated several thousand times at the 3' end of the DNA chain (total telomere length varies between 4 and 15 kb in humans). Telomere length in the majority of human somatic cells decreases by 20–200 base pairs during one cell division (Saliques et al., 2010). Telomeres preserve the integrity and stability of genomes during replication (Blackburn, 2001). Cells with critically short telomeres undergo chromosomal end-to-end fusions, replicative senescence and apoptosis. Overly short telomeres lead to a decrease of functional cells, which contributes to overall tissue and organ dysfunction (Wong et al., 2010). Telomere synthesis occurs via the influence of enzymatic complexes of telomerase. In contrast to somatic cells, germ, stem and tumour cells as well as immortalised cell lines maintain high levels of telomerase activity. Therefore, they display longer telomeres and maintain a relatively high proliferative potential (Liu et al., 1999; Collins, 2000; Houben et al., 2008). Telomere length is closely connected with the cellular cycle and replicative ageing (Edo et al., 2005). Telomere length differs between individuals of the same age and also between the different

tissues of identical subjects (Dlouha et al., 2014); this dissimilarity already exists after birth. Oestrogen-dependent activation of telomerase may contribute to the gender-related differences in telomere length that arise during adulthood (Fuster and Andrés, 2006). Environmental factors such as oxidative stress, smoking and UV radiation contribute to the reduction of telomere length (Butt, 2010; Wong et al., 2010).

Ageing is a major non-modifiable cardiovascular risk factor. During ageing of the human organism the heart is subject to functional, morphological and cellular changes. Ageing by itself needs not necessarily lead to heart failure, but it is probable that cellular changes associated with ageing decrease the threshold of the onset of heart failure symptoms. Both cardiovascular risk factors and common cardiovascular diseases such as atherosclerosis, heart failure and hypertension are associated with short leukocyte telomeres, but the causality remains undetermined (van der Harst et al., 2007; Fyhrquist et al., 2013).

There exist a number of complications in patients after heart transplantation that are understood as being potentially associated with the telomere length. The reasons for the inter-individual heterogeneities in survival time after heart transplantation are still incompletely understood, but biological ageing is one of the possible contributors (Harley, 1991). Leukocyte telomere length (LTL) of donors is associated with survival in patients with allogeneic haematopoietic cell transplantation (Gadalla et al., 2015). Shorter LTL may predict worse survival in idiopathic pulmonary fibrosis (Stuart et al., 2014) or chronic graft dysfunction after kidney transplantation (Oetting et al., 2014). Thus far, no study has focused on monitoring the correlation between LTL and survival time (ST) in patients after heart allograft transplantation.

The aim of our study was to verify the hypothesis that LTL can predict the ST of patients after heart allograft transplantation.

Material and Methods

Subjects

One hundred and twenty seven patients (20 women; age 39.9 ± 13.7 years, and 107 men; age 44.9 ± 9.6 years) who underwent orthotopic heart transplantation in the period from January 1984 to August 1997 were included in the study and their blood samples were collected between 1994 and 1997 at the Institute for Clinical and Experimental Medicine in Prague (Vancura et al., 1999). The protocol of this study was carried out according to the principles of the Declaration of Helsinki. All examined individuals gave their informed signed consent, which, together with the protocol of the study, were approved by the institute's ethics committee.

DNA analysis, measurement of telomere length

Genomic DNA was extracted from whole blood (Miller et al., 1988). We analysed the telomere length as de-

scribed in detail previously (Dlouha et al., 2012). The analysis was performed in the Rotor-Gene 3000 (Corbett Research Ltd, Sydney, Australia) using a quantitative polymerase chain reaction (qPCR)-based method with slight modifications (Cawthon, 2002; Salpea et al., 2008). The relative telomere length was calculated as the ratio of telomere repeats to a single-copy gene (SCG) (T/S ratio). The acidic ribosomal phosphoprotein PO (36B4) gene was selected as the SCG.

Statistical analysis

Analyses were performed with JMP 10 statistical software. Normal distribution of LTL data was examined by the Shapiro-Wilk W test. A comparison between two groups was performed with the Student's t-test. We used Cox proportional hazards models (adjusting for age and sex) to examine the association between survival and diagnosis of heart failure or gender. A linear regression model was used to evaluate the association between LTL and age. P values less than 0.05 were considered significant. Results are expressed as means ± SD (or SE when comparing LTL between the subgroups of patients).

Results

General characteristics of the patients

Basic characteristics of the analysed subjects are summarised in Table 1. The average age of heart recipients and donors was almost identical (P = 0.76). Over the 30-year follow up, 89 transplanted patients died. Maximal ST was 23 years and minimal one month. Noncardiac causes of death (mostly malignancy, sepsis, renal failure or multi-organ failure) were detected in 54 patients. Cardiac causes of death were found in 28 subjects, and in the remaining seven subjects the cause of death was unclear (Table 2). Patients with CAD were slightly older (Table 3) in comparison with subjects with DCM (P < 0.04).

The mean survival time of heart recipients was significantly associated with CAD diagnosis (RR = 1.6, 95% CI = 1.01–2.55; Fig. 1A) but not affected by the gender of the recipients (RR = 1.6, 95% CI = 0.92–3.25; Fig. 1B).

Table 1. Summary characteristics of the study group. Age, survival time and leukocyte telomere length are expressed as arithmetical means ± SD.

N	127
Recipient age (years)	44.1 ± 10.5
Donor age (years)	43.8 ± 9.6
Recipient gender (males/ females)	107/20

Aetiology of heart failure:
    dilated cardiomyopathy
    coronary artery disease
    other
	85
	35
	7

Survival time of deceased recipients (years)	13.5 ± 6.4
Unadjusted leukocyte telomere length	0.95 ± 0.2

Table 2. Causes of death in transplanted patients

Cause of death	N
Cardiovascular diseases	28
Oncologic diseases	20
Infections	11
Renal failure	7
Multi-organ failure	5
Cerebrovascular diseases	2
Abdominal aortic aneurism	1
Liver failure	2
Bronchopneumonia	2
Accidents	2
Other	2
Unknown reasons	7

Analysis of leukocyte telomere length

As expected, LTL was inversely associated with the age of heart recipients ($P = 0.0001$; Fig. 2A). Within the patient groups, cases with CAD had shorter LTL in comparison with cases with DCM ($P < 0.005$ and $P < 0.05$ after adjustment for age, Table 3).

We did not find any significant correlation between LTL and survival time in patients after heart transplantation ($P = 0.40$ after adjustment for age). When analysed in more detail, cases of non-cardiac cause of death had significantly shorter LTL ($P < 0.008$, $P < 0.05$ after adjustment for age) than patients with cardiovascular (CV)

Table 3. Comparison of age and LTL in subgroups of heart recipients according to mortality events and aetiology of HF. Data are expressed as means ± SD.

	N	Age	P	Leukocyte telomere length[#]	P*
DCM/CAD	85/35	$43.0 \pm 11.0/47.2 \pm 9.4$	< 0.04	$0.96 \pm 0.02/0.88 \pm 0.03$	< 0.05
Survivors/non-survivors	38/89	$43.8 \pm 12.4/44.3 \pm 9.7$	ns	$0.93 \pm 0.04/0.95 \pm 0.02$	ns
Mortality CV/non-CV events	28/54	$39.7 \pm 12.5/46.4 \pm 7.4$	< 0.004	$1.02 \pm 0.04/0.93 \pm 0.03$	< 0.05

[#]denotes SE; *denotes P after adjustment for age
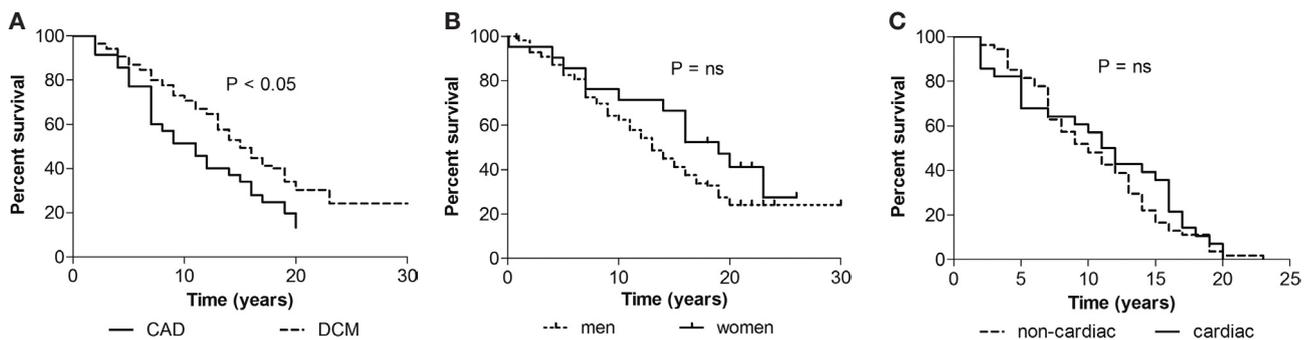
Fig. 1. Kaplan-Maier survival analysis comparing the survival time of recipients according to **A**) heart failure aetiology: CAD (N = 35) and DCM (N = 85); **B**) gender: men (N = 107) and women (N = 20); and **C**) cardiac (N = 28) or non-cardiac (N = 54) cause of death. P values are adjusted for age.
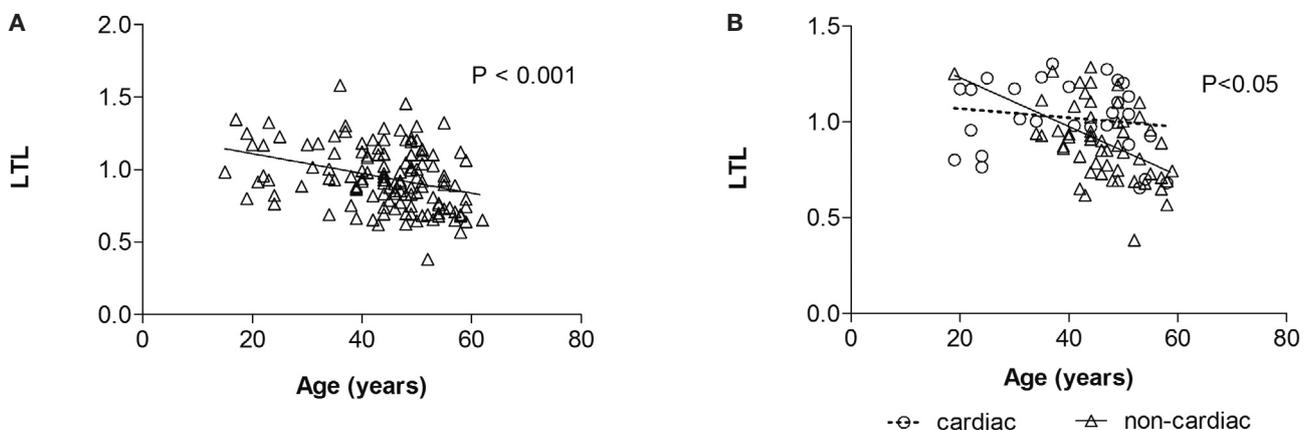
Fig. 2. Correlation between leukocyte telomere length and age in **A**) all analysed heart recipients and **B**) in subgroups of patients with different causes of death

cause of death (Table 3). Moreover, although there was a different trend in LTL association with age between the subjects with CV and subjects with non-CV causes of death (Fig. 2B), the survival time did not differ between these two subgroups (11.2 ± 1.2 years vs. 10.4 ± 0.8 years; $P = 0.56$ after adjustment for age; Fig. 1C).

Discussion

Our study is the first to focus on a possible association between leukocyte telomere length and survival time in heart transplant recipients. We did not find any significant association between the telomere length and the survival time of heart transplant recipients over 30 years of follow-up.

Patients with heart failure who have undergone transplantation suffer from many serious diseases, which can contribute to or affect telomere attrition. Also, immunosuppressive treatment used by all these patients can significantly influence the telomere shortening (Welzl et al., 2014).

Recently, a growing interest in cardiovascular ageing has led to a number of studies investigating LTL and its associations with various diseases (Nilsson et al., 2013). Shorter LTL has been reported in association with atherosclerosis, myocardial infarction, type 2 diabetes mellitus and all-cause mortality (Farzaneh-Far et al., 2008; Maubaret et al., 2010; Aviv, 2012; Zhang et al., 2013). It has been shown that patients with heart failure have shorter leukocyte telomeres compared with control subjects of the same age and sex (van der Harst et al., 2007). One study detected an association between telomere attrition and age related to left ventricular diastolic dysfunction (Akhasheva et al. 2015). Controversially, there are also studies that do not support a linear association between LTL and cardiovascular heart disease (Ye et al., 2013; Cui et al., 2014).

Also, the frequent studies that focus on leukocyte telomere length and general survival are controversial and no definitive conclusion can be drawn (Cawthon et al., 2003; Bischoff et al., 2006; Harris et al., 2006; Epel et al., 2009; Njajou et al., 2009; Astrup et al., 2010; Fitzpatrick et al., 2011; Strandberg et al., 2011; Honig et al., 2012).

Our study has several limitations. The fact that leukocyte DNA was only used for the analysis and because samples were not collected immediately at the same time after transplantation may have potentially affected the obtained results. Further, heart transplantation subjects represent a very distinct group of patients (mainly, but not only, the severity of their diagnosis needs to be taken into account) and it is not possible to collect thousands of cases, as is usually required in the case of "traditional" genetic studies. Finally, these patients usually spend years on strong medical support, which can also influence the telomere length per se, as generally suggested previously (Chkhotua et al., 2005).

Although we determined the telomere length, it is not the only critical aspect to telomere maintenance. Measures were performed in leukocytes but not in myocardial tissue. Telomerase activity was not analysed because the DNA samples were collected a long time before the study was designed and no further stored samples were available. This was a retrospective analysis of a unique group of patients who had to use long-term immunosuppressive drugs. It should also be noted that telomere length does not satisfy the strict criteria for being an exclusive biomarker of ageing, but adds predictive power to that of chronological age, and can be considered a marker of cardiovascular ageing.

Conclusions

We did not confirm the assumption that leukocyte telomere length would be a strong predictor of long-time survival in patients after heart transplantation. We suggest, however, that lower leukocyte telomere length may be a predictor of non-cardiac mortality in these patients.

Disclosure of conflict of interest

The authors confirm that they do not have any conflicts of interest.

References

Akasheva, D. U., Plokhova, E. V., Tkacheva, O. N., Strazhesko, I. D., Dudinskaya, E. N., Kruglikova, A. S., Pykhtina, V. S., Brailova, N. V., Pokshubina, I. A., Sharashkina, N. V., Agaltsov, M. V., Skvortsov, D., Boytsov, S. A. (2015) Age-related left ventricular changes and their association with leukocyte telomere length in healthy people. *PLoS One* **10**, e0135883.

Astrup, A. S., Tarnow, L., Jorsal, A., Lajer, M., Nzietchueng, R., Benetos, A., Rossing, P., Parving, H. H. (2010) Telomere length predicts all-cause mortality in patients with type 1 diabetes. *Diabetologia* **53**, 45-48.

Aviv, A. (2012) Genetics of leukocyte telomere length and its role in atherosclerosis. *Mutat. Res.* **730**, 68-74.

Bischoff, C., Petersen, H. C., Graakjaer, J., Andersen-Ranberg, K., Vaupel, J. W., Bohr, V. A., Kolvraa, S., Christensen, K. (2006) No association between telomere length and survival among the elderly and oldest old. *Epidemiology* **17**, 190-194.

Blackburn, E. H. (2001) Switching and signaling at the telomere. *Cell* **106**, 661-673.

Butt, H. Z., Atturu, G., London, N. J., Sayers, R. D., Bown, M. J. (2010) Telomere length dynamics in vascular disease: a review. *Eur. J. Vasc. Endovasc Surg.* **40**, 17-26.

Cawthon, R. M. (2002) Telomere measurement by quantitative PCR. *Nucleic Acids Res.* **30**, e47.

Cawthon, R. M., Smith, K., O'Brien, E., Sivatchenko, A., Kerber, R. (2003) Association between telomere length in blood and mortality in people aged 60 years or older. *Lancet* **361**, 393-395.

Chkhotua, A. B., Schelzig, H., Wiegand, P., Grosse, S., Reis, S., Art, M., Abendroth, D. (2005) Influence of ischaemia/reperfusion and LFA-1 inhibition on telomere lengths and CDKI genes in ex vivo haemoperfusion of primate kidneys. *Transpl. Int.* **17**, 692-698.

Collins, K. (2000) Mammalian telomeres and telomerase. *Curr. Opin. Cell Biol.* **12**, 378-383.

Cui, G., Sun, J., Zhang, L., Li, R., Wang, Y., Cianflone, K., Ding, H., Wang, D. W. (2014) Lack of causal relationship between leukocyte telomere length and coronary heart disease. *Atherosclerosis* **233**, 375-380.

Dlouha, D., Pitha, J., Lanska, V., Hubacek, J. A. (2012) Association between FTO 1st intron tagging variant and telomere length in middle aged females. 3PMFs study. *Clin. Chim. Acta* **413,** 1222-1225.

Dlouha, D., Maluskova, J., Kralova Lesna, I., Lanska, V., Hubacek, J. A. (2014) Comparison of the relative telomere length measured in leukocytes and eleven different human tissues. *Physiol. Res.* **63 (Suppl 3),** S343-S350.

Edo, M. D., Andrés, V. (2005) Aging, telomeres, and atherosclerosis. *Cardiovasc. Res.* **66**, 213-221.

Epel, E., Merkin, S. S., Cawthon, R., Blackburn, E. H., Adler, N. E., Pletcher, M. J., Seeman, T. E. (2009) The rate of telomere shortening predicts mortality from cardiovascular disease in elderly men. *Aging (Albany NY)* **1**, 81-88.

Farzaneh-Far, R., Cawthon, R. M., Na, B., Browner, W. S., Schiller, N. B., Whooley, M. A. (2008) Prognostic value of leukocyte telomere length in patients with stable coronary artery disease: data from the Heart and Soul Study. *Arterioscler. Thromb. Vasc. Biol.* **28**, 1379-1384.

Fitzpatrick, A. L., Kronmal, R. A., Kimura, M., Gardner, J. P., Psaty, B. M., Jenny, N. S., Tracy, R. P., Hardikar, S., Aviv, A. (2011) Leukocyte telomere length and mortality in the Cardiovascular Health Study. *J. Gerontol. A Biol. Sci. Med. Sci.* **66**, 421-429.

Fuster, J. J., Andrés, V. (2006) Telomere biology and cardiovascular disease. *Circ. Res.* **99**, 1167-1180.

Fyhrquist, F., Saijonmaa, O., Strandberg, T. (2013) The roles of senescence and telomere shortening in cardiovascular disease. *Nat. Rev. Cardiol.* **10**, 274-283.

Gadalla, S. M., Wang, T., Haagenson, M., Spellman, S. R., Lee, S. J., Williams, K. M., Wong, J. Y., De Vivo, I., Savage, S. A. (2015) Association between donor leukocyte telomere length and survival after unrelated allogeneic hematopoietic cell transplantation for severe aplastic anemia. *JAMA* **313**, 594-602.

Harley, C. B. (1991) Telomere loss: mitotic clock or genetic time bomb? *Mutat. Res.* **256**, 271-282.

Harris, S. E., Deary, I. J., MacIntyre, A., Lamb, K. J., Radhakrishnan, K., Starr, J. M., Whalley, L. J., Shieleys, P. G. (2006) The association between telomere length, physical health, cognitive ageing, and mortality in non-demented older people. *Neurosci. Lett.* **406**, 260-264.

Honig, L. S., Kang, M. S., Schupf, N., Lee, J. H., Mayeux, R. (2012) Association of shorter leukocyte telomere repeat length with dementia and mortality. *Arch. Neurol.* **23**, 1-8.

Houben, J. M., Moonen, H. J., van Schooten, F. J., de Boer, R. A., (2008) Telomere length assessment: biomarker of chronic oxidative stress? *Free Radic. Biol. Med.* **44**, 235-246.

Liu, K., Schoonmaker, M. M., Levine, B. L., June, C. H., Hodes, R. J., Weng, N. P. (1999) Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. *Proc. Natl. Acad. Sci. USA* **96**, 5147-5152.

Maubaret, C. G., Salpea, K. D., Jain, A., Cooper, J. A., Hamsten, A., Sanders, J., Montgomery, H., Neil, A., Nair, D., Humphries, S. E. (2010) Telomeres are shorter in myocardial infarction patients compared to healthy subjects: correlation with environmental risk factors. *J. Mol. Med. (Berl)* **88**, 785-794.

Miller, S. A., Dykes, D. D., Polesky, H. F. (1988) A simple salting out procedure for DNA extraction from human nucleated cells. *Nucleic Acids Res.* **16,** 1215.

Nilsson, P. M., Tufvesson, H., Leosdottir, M., Melander, O. (2013) Telomeres and cardiovascular disease risk: an update 2013. *Transl. Res.* **162**, 371-380.

Njajou, O. T., Hsueh, W. C., Blackburn, E. H., Newman, A. B., Wu, S. H., Li, R., Simonsick, E. M, Cumming, S. R., Cawthon, R. M. (2009) Association between telomere length, specific causes of death, and years of healthy life in health, aging, and body composition, a population-based cohort study. *J. Gerontol. A Biol. Sci. Med. Sci.* **64**, 860-864.

Oetting, W. S., Guan, W., Schladt, D. P., Wildebush, W. A., Becker, J., Thyagarajan, B., Jacobson, P. A., Matas, A. J., Israni, A. K. (2014) Telomere length of recipients and living kidney donors and chronic graft dysfunction in kidney transplants. *Transplantation* **97**, 325-329.

Saliques, S., Zeller, M., Lorin, J., Lorgis, L., Teyssier, J. R., Cottin, Y., Rochette, L., Vergely, C. (2010) Telomere length and cardiovascular disease. *Arch. Cardiovasc. Dis*. **103**, 454-459.

Salpea, K. D., Nicaud, V., Tiret, L., Talmud, P. J., Humphries, S. E., EARS II group. (2008) The association of telomere length with paternal history of premature myocardial infarction in the European Atherosclerosis Research Study II. *J. Mol. Med. (Berl)* **86**, 815-824.

Strandberg, T. E., Saijonmaa, O., Tilvis, R. S., Pitkala, K. H., Strandberg, A. Y., Miettinen, T. A., Fyhrquist, F. (2011) Association of telomere length in older men with mortality and midlife body mass index and smoking. *J. Gerontol. A Biol. Sci. Med. Sci.* **66**, 815-820.

Stuart, B. D., Lee, J. S., Kozlitina, J., Noth, I., Devine, M. S., Glazer, C. S., Torres, H. F., Kaza, V., Girod, C. E., Jones, K. D., Elicker, B. M., Ma, S. F., Vij, R., Collard, H. R., Wolters, P. J., Garcia, C. K. (2014) Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *Lancet Respir. Med*. **2**, 557-565.

van der Harst, P., van der Steege, G., de Boer, R. A., Voors, A. A., Hall, A. S., Mulder, M. J., van Gilst, W. H., van Veldhuisen, D. J.; MERIT-HF Study Group (2007) Telomere length of circulating leukocytes is decreased in patients with chronic heart failure. *J. Am. Coll. Cardiol.* **49**, 1459-1464.

Vancura, V., Hubacek, J., Malek, I., Gebauerova, M., Pitha, J., Dorazilova, Z., Langova, M., Zelizko, M., Poledne, R. (1999) Does angiotensin-converting enzyme polymorphism influence the clinical manifestation and progression of heart failure in patients with dilated cardiomyopathy? *Am. J. Cardiol.* **83,** 461-462.

Welzl, K., Kern, G., Mayer, G., Weinberger, B., Säemann, M. D., Sturm, G., Grubeck-Loebenstein, B., Koppelstaetter, C. (2014) Effect of different immunosuppressive drugs on immune cells from young and old healthy persons. *Gerontology* **60**, 229-238.

Wong, L. S., van der Harst, P., de Boer, R. A., Huzen, J., van Gilst, W. H., van Veldhuisen, D. J. (2010) Aging, telomeres and heart failure. *Heart Fail. Rev.* **15**, 479-486.

Ye, S., Shaffer, J. A., Kang, M. S., Harlapur, M., Muntner, P., Epel, E., Guernsey, D., Schwartz, J. E., Davidson, K. W., Kirkland, S., Honig, L. S., Shimbo, D. (2013) Relation between leukocyte telomere length and incident coronary heart disease events (from the 1995 Canadian Nova Scotia Health Survey). *Am. J. Cardiol.* **111**, 962-967.

Zhang, W., Chen, Y., Wang, Y., Liu, P., Zhang, M., Zhang, C., Hu, F. B., Hui, R. (2013) Short telomere length in blood leucocytes contributes to the presence of atherothrombotic stroke and haemorrhagic stroke and risk of post-stroke death. *Clin. Sci. (Lond)* **125**, 27-36.